

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12Q 1/68, A61K 39/00, 37/02	A1	(11) International Publication Number: WO 93/16199
		(43) International Publication Date: 19 August 1993 (19.08.93)

(21) International Application Number: PCT/US93/01231

(22) International Filing Date: 11 February 1993 (11.02.93)

(30) Priority data:
07/836,642 14 February 1992 (14.02.92) US(71)(72) Applicant and Inventor: REED, Steven, G. [US/US];
2843 122nd Place N.E., Bellevue, WA 98005 (US).(74) Agent: PERKINS, Patricia, Anne; Immunex Corporation,
51 University Street, Seattle, WA 98101 (US).(81) Designated States: BB, BR, CA, JP, KP, KR, LK, MG,
MW, SD, European patent (AT, BE, CH, DE, DK, ES,
FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI pa-
tent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN,
TD, TG).**Published***With international search report.*(54) Title: PEPTIDE FOR DIAGNOSING AND IMMUNIZING AGAINST *T. cruzi* INFECTION

(57) Abstract

There is disclosed an antigenic peptide that comprises at least 15 amino acids having the sequence Ala Glu Pro Lys X Ala Glu Pro Lys X Ala Glu Pro Lys X, wherein X is Pro or Ser. This peptide is useful in an ELISA assay to detect antibodies specific to *T. cruzi* infection and Chagas disease. This peptide is further useful in a vaccine composition for immunizing an individual to prevent Chagas disease upon exposure to *T. cruzi*.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TC	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	MI	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

TITLE

5 Peptide For Diagnosing and Immunizing Against *T. cruzi* Infection

Technical Field of the Invention

10

The present invention relates to a 15 mer peptide that is the epitope repeat sequence for a predominant antigen of *T. cruzi*. The inventive peptide is useful for diagnosing *T. cruzi* infection and for use in a vaccine to immunize an individual to reduce *T. cruzi* infection and clinical manifestations of Chagas disease.

15

Background of the Invention

Chagas disease is one of the most important endemic problems in Central and South America, for which no definitive chemotherapeutic or immunological treatment is available. *Trypanosoma cruzi* (*T. cruzi*) is the agent of Chagas disease. Infection with a protozoan parasite *T. cruzi*, the causative agent of Chagas disease, occurs in an estimated 18 million persons throughout Latin America and is a major cause of chronic heart disease. Immune responses after *T. cruzi* infection are particularly complex due to the biochemical diversity of multiple parasite strains and influence of host-genetic factors. The result is a wide diversity in clinical manifestations of Chagas disease and, in some cases, the disruption of immune regulation leading to immunosuppression and/or development of autoimmunity. This parasite has a complex life cycle involving an epimastigote stage in the insect vector and two main stages in the mammalian host. One stage is present in blood (trypomastigote) and a second stage is intracellular (amastigote).

30

The acute phase of *T. cruzi* infection is often asymptomatic. The infection may remain quiescent for decades. Some patients may, however, develop a progressive chronic form of the disease with cardiac and/or digestive tract alterations. After the acute phase with parasitemia, parasite growth is usually controlled by the host and patients or animals enter into a chronic phase where few parasites are present in the blood.

35

Immune responses to protozoan infection are complex, involving both humoral and cell-mediated responses to an array of parasite antigens. Infection often involves multiple life cycle stages of these parasites, which adds to the diversity of antigens potentially important for the development of protective immunity. To examine the

40

molecular basis of the immune responses elicited during these infections, recent efforts have focused on evaluating responses to defined parasite B- and T-cell epitopes.

5 *T. cruzi* infections are often subtle and long-lasting, making diagnosis crucial and problematic. Detecting antibodies against parasite antigens is a most common and reliable method of determining clinical and subclinical infections. Presently, serological tests use whole or lysed *T. cruzi* and require positive results on two of three tests, including complement fixation, indirect immunofluorescence, passive agglutination, or ELISA to accurately detect *T. cruzi* infection. The expense as well as difficulty in performing such tests reliably prevent the screening of blood or sera in many endemic areas.

10 Blood bank screening is particularly important in South America, where 0.1-62% of samples may be infected and where the parasite is frequently transmitted by blood transfusion. It is also important and of increasing concern that the blood supply in certain U.S. cities is contaminated with *T. cruzi* parasites.

15 Therefore, there is a need in the art for a greater understanding of responses to specific parasite antigens. Although several antigens of *T. cruzi* have been identified and characterized biochemically, limited data are available on the evaluation of human immune responses to these molecules.

20 Summary of the Invention

The present invention relates to the cloning and expression of a *T. cruzi* antigen gene sequence encoding the immunodominant protein with an essential repetitive epitope. This gene sequence is conserved among diverse *T. cruzi* isolates. The inventive antigenic peptide domain of *T. cruzi* is predominantly expressed by trypanomastigotes, the infective form of the parasite. Evaluation of human immune responses to this antigenic peptide domain of *T. cruzi* revealed easily detectable levels of antibodies in greater than 95 percent of *T. cruzi* infected sera samples from several South American countries.

30 The antigenic peptide domain of *T. cruzi* comprises the amino acid sequence Ala Glu Pro Lys X₁ Ala Glu Pro Lys X₂ Ala Glu Pro Lys X₃, wherein X is Pro or Ser and when X₁ is Ser, X₃ is Ser, or when X₁ is Pro, X₃ is Pro. The antigenic peptide can also comprise a linker sequence at either the N-terminus or the C-terminus of the antigenic peptide domain wherein the linker sequence facilitates attachment or conjugation of the antigenic peptide domain to various carrier molecules or solid support systems.

The present invention further comprises a method for diagnosing Chagas disease or *T. cruzi* infection by detecting antibodies specific to the inventive antigenic peptide domain. This method comprises contacting a sample of whole blood or an immunoglobulin-containing component of whole blood with the inventive antigenic peptide conjugated to a solid phase, washing unbound antibodies from the solid phase, adding the inventive antigenic peptide conjugated to a detectable moiety to form an antigenic peptide-antibody complex, and detecting the antigenic peptide-antibody complex.

Further still, the present invention comprises a vaccine composition for immunizing an individual for preventing Chagas disease symptoms of *T. cruzi* infection upon exposure to *T. cruzi*. The vaccine composition comprises an immunologically effective amount of the inventive antigenic peptide and a vaccine adjuvant, such as Freund's adjuvant.

Brief Description of the Drawings

Figure 1 shows the DNA sequence and deduced amino acid sequence of a 636 base pair TcD insert shown with residues blocked to indicate a 10-amino acid repetitive unit and the number of repeats. Boxed amino acids mark degeneracies in the repeat unit.

Figures 2A and 2B show an ELISA evaluation of recombinant TcD and synthetic TcD peptide. Absorbance values are based upon a population of 127 individuals with *T. cruzi* infection, 34 individuals with leishmaniasis, 10 with malaria, 17 mycobacterial infections and 32 normal sera, against *T. cruzi* lysate (hatched bars) and recombinant antigenic peptide (double dash hatched bars). In Figure 2B the adsorbance values are for 127 *T. cruzi* infection sera, 9 acute Chagas disease sera, 15 other infected sera, including leishmaniasis, 10 malaria, 16 mycobacterial infection and 32 normal sera. All sera samples were evaluated with synthetic antigenic peptide.

Detailed Description of the Invention

We have identified and synthesized a major antigenic epitope of an approximately 260kD *T. cruzi* antigen expressed predominantly by trypomastigotes. This antigenic peptide domain of *T. cruzi* is conserved among geographically diverse *T. cruzi* isolates. Conservation of the antigen was further indicated by the presence of TcD-specific antibodies in sera from Chagas patients having great clinical and geographical diversity, produced as a result of natural infection with *T. cruzi* parasites

expressing the antigenic peptide repetitive epitope. This antigenic peptide domain of *T. cruzi* is useful for diagnosing *T. cruzi* infection, Chagas disease, and for use in a vaccine composition to protect individuals from Chagas disease or other lethal complications upon exposure to *T. cruzi* parasites.

- 5 Response to the antigenic peptide domain of *T. cruzi* was found to have such extremely high prevalence in seroreactive Chagas patients that it has usefulness as a diagnostic agent. Particularly, the antigenic peptide domain induces antibodies in such patients such that infection by *T. cruzi* or symptoms of Chagas disease can be diagnosed by the presence of such antibodies using an ELISA-type diagnostic assay with
10 recombinant or synthetic antigenic peptide from *T. cruzi*.

The antigenic peptide domain of *T. cruzi* comprises the protein sequence Ala Glu Pro Lys X₁ Ala Glu Pro Lys X₂ Ala Glu Pro Lys X₃, wherein X is Pro or Ser and when X₁ is Ser, X₃ is Ser, or when X₁ is Pro, X₃ is Pro. The antigenic peptide comprises at least a 15 residue sequence having three groups of five amino acids as a
15 repeat sequence. The antigenic peptide domain can have additional five residue Ala Glu Pro Lys X sequences to cause the antigenic peptide to be 15, 20, 25, 30, 35, 40, etc., amino acids in length.

- Moreover, mice immunized with the inventive antigenic peptide have been protected from lethal *T. cruzi* infection. Therefore, the inventive antigenic peptide can
20 be used as a vaccine to prevent generalized infection from *T. cruzi* and enhance the host immune response to *T. cruzi* exposure.

- The present inventive antigenic peptide is the first such antigen having an epitope with serodiagnostic potential. A previously reported cloned *T. cruzi* antigen (Ibanez et al., *Mol. Biochemical Parasitology* 25:175, 1987; Ibanez et al., *Mol.*
25 *Biochemical Parasitology* 30:27, 1988; and Affranchino et al., *Mol. Biochemical Parasitology* 34:221, 1989) contained repetitive domains, with one domain reportedly present in an 85kD antigen. Although the 5-amino acid repeat sequence in the 85kD antigen is similar to the second half of the repeat sequence of the inventive antigenic peptide domain, reactivity with the 85kD antigen demonstrated only 40% positives in
30 Chagas patient sera. The present inventive peptide, by contrast, exhibited confirmation rates of greater than 95%. This result is consistent with our mapping studies, which indicated that peptides containing only the 5-residue repeat sequence lacked an essential portion of a dominant B-cell epitope of TcD.

- In ELISA assay with the synthetic antigenic peptide domain according to the
35 present invention is easy to perform, allows for standardization of reagents, permits screening of large numbers of samples, and can be used with either blood or serum samples.

We purified a series of *T. cruzi* antigens to find an antigenic peptide domain of a *T. cruzi* antigen responsible for uniform epitope binding in a vast majority of *T. cruzi* infected individuals. We made a genomic expression library in λ ZAPII with mechanically sheared DNA of *T. cruzi*. Recombinants expressing *T. cruzi* antigen genes were selected based upon their reactivity with a pool of Chagas patients' sera, preadsorbed to remove anti-*E. coli* reactivity. Of twelve clones identified, one clone, called TcD, was exceptionally reactive with the pooled patients' sera. Purified recombinant antigen of clone TcD migrated at about 59kD on SDS/PAGE. In an immunoblot analysis, the TcD antigen was strongly recognized by pooled Chagas patient sera but not recognized by a pool of normal sera obtained from normal volunteers in Seattle, Washington. Moreover, a pool of high-titer sera from patients with acute visceral leishmaniasis, an infection known to induce antibodies cross-reactive with *T. cruzi*, was negative for the TcD antigen.

The sequence of the TcD antigen is shown in Figure 1. Clone TcD encodes a 10-amino acid repetitive sequence. DNA sequence analysis of clone TcD predicted an amino acid sequence comprised entirely of a 10-amino acid repeat sequence arrayed in tandem, and present in 20.5 copies with minor degeneracies in 5 positions (Figure 1). The predicted molecular weight of recombinant, unglycosylated TcD antigen was 36.3kD. The migration of the TcD antigen at 59kD observed during SDS/PAGE most likely reflected a high proline content (28%).

A 636 base pair insert of clone TcD was used to probe Southern blots of *T. cruzi* DNA and DNA from several other protozoan parasites of humans. The probe hybridized to multiple restriction fragments of *T. cruzi* DNA but not to the other protozoan parasites including *T. brucei*, *Leishmania chagasi*, *L. amazonensis*, *L. donovani*, and *T. rangeli*. Analysis of DNA from seven geographically diverse *T. cruzi* isolates indicate that TcD gene sequence was conserved among isolates showing restriction fragment length polymorphism and variability in gene-copy number.

The antigenic peptide of *T. cruzi* comprises at least 15 amino acids having the sequence Ala Glu Pro Lys X Ala Glu Pro X Ala Glu Pro Lys X, wherein X is Pro or Ser. Additional 5 amino acid sequences (Ala Glu Pro Lys X) may be added to the basic 15 residue antigenic peptide domain of *T. cruzi*. A further sequence may be added to the antigenic peptides to link this peptide at either its N-terminal or C-terminal wherein the linker sequence facilitates attachment or conjugation of the antigenic peptide to carrier molecules. An example of a linker sequence is Gly Cys Gly. The antigenic peptide is made, preferably, by synthetic means on a programmable peptide synthesizer.

ELISA assays have been conducted utilizing antigens or antibodies as the outer components of a sandwich. An ELISA assay of blood or sera from individuals can detect *T. cruzi* infection of Chagas disease by an antibody specific to the antigenic peptide domain. Therefore, one component of an ELISA sandwich comprises the antigenic peptide of the present invention. Another component comprises an agent that can bind to the anti-*T. cruzi* antibody include, for example, anti-immunoglobulin or protein A. Each component can form a antigenic peptide-antibody complex which contains a detectable moiety. The detectable moiety is known in the art of ELISA diagnostic assays as that component that identifies the antigenic peptide-antibody complex through visual, fluorescent, radionuclide or other means. Common examples of detectable moieties include fluorescent or chemiluminescent agents or enzymes such as horseradish peroxidase.

In a series of studies, patient sera from *T. cruzi* infected individuals or Chagas patients was compared with sera from patients infected with other parasites or normal sera. Patient sera with ELISA values at least five standard deviations greater than mean adsorbance value of normal controls were considered positive. Of confirmed *T. cruzi* infected sera, greater than 95 percent (121 of 127) were positive for an anti-TcD antibody. Therefore, detection of an anti-TcD antibody in *T. cruzi* infected individuals is a reliable method of detecting Chagas disease or *T. cruzi* infection.

Example 1

This example illustrates cloning of the TcD antigen from *T. cruzi*. A genomic library was constructed in λ ZAPII (Stratagene) with mechanically sheared DNA of *T. cruzi*. Construction of the library and excision of a pBFLK (-) phagemid sequences were performed according to manufacturer's protocols. Recombinants were screened with a pool of Chagas patients' sera preadsorbed to remove anti-*E. coli* reactivity as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. A 59kD recombinant antigen, called clone TcD, was purified from a soluble lysate of induced bacterial cultures by ammonium sulfate fractionation, preparative isoelectric focusing with a Bio-Rad Rotofor IEF cell and 1% - 3/10 ampholytes in the presence of 8 M urea followed by SDS/PAGE and electroelution as described in Reed et al., *J. Clin. Invest.* 85:690, 1990. Protein concentrations were determined using a Pierce BCA protein assay.

Patients' sera were collected from well-characterized patients with acute or chronic Chagas disease or indeterminant *T. cruzi* infection from the South American countries Brazil (Northern and Southern), Bolivia and Argentina. Normal sera were

obtained from individuals living in non-endemic *T. cruzi* areas (Seattle). Sera from confirmed visceral or cutaneous leishmaniasis were obtained from parasitologically confirmed Sudanese patients. Mycobacterial infection sera were obtained from Seattle (tuberculosis) or Haiti for leprosy.

5 Twelve clones were identified. One clone, called TcD, was exceptionally reactive with patients' sera. Purified recombinant antigen from clone TcD migrated at 59kD on SDS/PAGE. An immunoblot analysis found that the TcD antigen was strongly recognized by Chagas patients' serum but not recognized by normal sera or by high-titer sera from patients with acute visceral leishmaniasis, an infection known to
10 induce antibodies cross-reactive with *T. cruzi*.

Example 2

This example illustrates an evaluation of synthetic antigenic peptides derived
15 from the antigenic peptide domain of *T. cruzi* having amino acid length of 5, 10, 15, and 20 amino acids in length. The synthetic peptides were constructed with five amino acid repeat sequences of Ala Glu Pro Lys X, wherein X is Pro or Ser. The data (Figure 2) show that a peptide containing 15 residues of the repeat sequence was required to map the immunodominant B-cell epitope of TcD. Moreover, the 15 amino
20 acid synthetic peptide had reactivity of patient sera comparable to that obtained with the recombinant molecule. One hundred sixteen of one hundred twenty Chagas sera patients gave positive adsorbance values. In addition, positive TcD-specific antibody responses were detected in 8 of 9 acute Chagas disease patients, indicative of an early immune response to *T. cruzi* infection to this epitope.

25

Example 3

This example illustrates the usefulness of the inventive antigenic peptide in a vaccine composition to reduce complication and mortality associated with *T. cruzi*
30 infection. A group of 8 week old female C57/6 mice (Jackson Labs, Bar Harbor, ME) were divided into three treatment groups. Group A (5 mice) was the control group and received no vaccine treatment. Group B was a second control group of 4 mice that received only adjuvant treatment. Group C (4 mice) received the inventive vaccine composition comprising the antigenic peptide (TcD) (15 mer) plus an adjuvant.

35 The treatment schedule was vaccine administration (s.c.) on day 0, wherein the vaccine comprised 200 µg TcD peptide in complete Freund's adjuvant. Vaccine was administered (i.p.) on day 24 comprising 100 µg TcD. Vaccine was also administered

(s.c.) on day 50 wherein this vaccine composition comprised 200 µg TcD peptide in incomplete Freund's adjuvant plus 25 µg muramyl dipeptide (MDP, Calbiochem) an additional adjuvant agent.

At day 57, each mouse was challenged with 1,000 *T. cruzi* (TcTc²) ip. At day 75 (or 18 days post challenge) peak parasitemia was determined and each animal was observed for mortality. The data presented in Table 1 below show the protective effects of the TcD peptide vaccine composition to *T. cruzi* challenge.

Table 1

10

<u>Group</u>	<u>Treatment</u>	<u>Peak Parasitemia</u>	<u>Mortality</u>
A	nothing	$4 \times 10^5 \pm 5 \times 10^5$	5/5
B	adjuvant	$4 \times 10^5 \pm 5 \times 10^5$	4/4
C	TcD + adjuvant	$1 \times 10^5 \pm 8 \times 10^4$	1/4

15

Claims

I claim:

- 5 1. An antigenic peptide domain of *T. cruzi* comprising the protein sequence Ala Glu Pro Lys X₁ Ala Glu Pro Lys X₂ Ala Glu Pro Lys X₃, wherein when X is Pro or Ser and when X₁ is Ser, X₃ is Ser or when X₁ is Pro, X₃ is Pro.
- 10 2. The peptide of claim 1 further comprising one or a plurality of Ala Glu Pro Lys X peptide sequences wherein X is Pro or Ser.
- 15 3. The peptide of claim 1 further comprising a linker sequence at either the N-terminal or the C-terminal, wherein the linker sequence facilitates attachment or conjugation of the antigenic peptide to carrier molecules.
- 20 4. An ELISA diagnostic assay to detect antibodies specific to *T. cruzi* infection in blood comprising:
 - (a) contacting a sample of whole blood on an immunoglobulin-containing component of whole blood with the antigenic peptide of claim 1 conjugated to a solid phase;
 - (b) washing unbound *T. cruzi*-specific antibodies from the solid phase;
 - (c) adding anti-immunoglobulin or Protein A conjugated to a detectable moiety to form an antigenic peptide-antibody complex; and
 - 25 (d) detecting the antigenic peptide-antibody complex.
- 30 5. The diagnostic assay of claim 4 wherein the detectable moiety is a colorometric agent, a fluorescent agent, a chemiluminescent agent or a radionuclide.
- 35 6. A method of detecting Chagas disease or *T. cruzi* infection comprising assaying a blood sample for an antibody that binds to the antigenic peptide of claim 1.
7. A vaccine composition for immunizing an individual to prevent Chagas disease upon exposure to *T. cruzi*, comprising an immunologically effective amount of the antigenic peptide of claim 1 and a vaccine adjuvant.

8. The vaccine composition of claim 7 wherein the vaccine adjuvant is a Freund's adjuvant.

1/2

GAATTCA

1

8 GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA GCA GAG CCT AAA CCA GCG GAG CCG AAA TCG
 ALA GLU PRO LYS PRO ALA GLU PRO LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

68 GCA GAG CCC AAA CCA GCG GAG CCG AAA TCG GCA GAG CCC AAA CCA GCG GAG CCG AAA TCA
 ALA GLU PRO LYS PRO ALA GLU PRO LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

128 GCG GGG CCT AAA CCA GCG GAG CCG AAG TCA GCG GAG CCT AAA CCA GCG GAG CCG AAA TCA
 ALA GLY PRO LYS PRO ALA GLY PRO LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

188 GCA GAG CCC AAA CCA GCG GAG CCG AAA TCG GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
 ALA GLU PRO LYS PRO ALA GLU PRO LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

248 GCA GAG CCC AAA CCA GCG GAG TCG AAG TCA GCA GAG CCT AAA CCA GCG GAG CCG AAA TCA
 ALA GLU PRO LYS PRO ALA GLU SER LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS SER

308 GCA GAG CCC AAA CCA GCG GAG TCG AAG TCA GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
 ALA GLU PRO LYS PRO ALA GLU SER LYS SER ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO

368 GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA GCA GAG CCC AAA CCA GCG GAG CCG AAA TCA
 ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO

428 GCG GAG CCC AAA CCA GCG GAG CCG AAA TCA GCA GAG CCC AAA CCA GCG GAG TCG AAA TCA
 ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU SER LYS PRO

488 GCG GGG CCT AAA CCA GCG GAG CCG AAG TCA GCG GAG CCA AAA CCA GCG GAG CCG AAA TCA
 ALA GLY PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO

548 GCG GAG CCA AAA CCA GCG GAG CCG AAA TCG GCA GAG CCC AAA CCA GCG GAG CCG AAG TCA
 ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO ALA GLU PRO LYS PRO

608 GCA GAG CCA AAA CCA GCG GAG CCGAATTC
 ALA GLU PRO LYS PRO ALA GLU

FIGURE 1

2/2

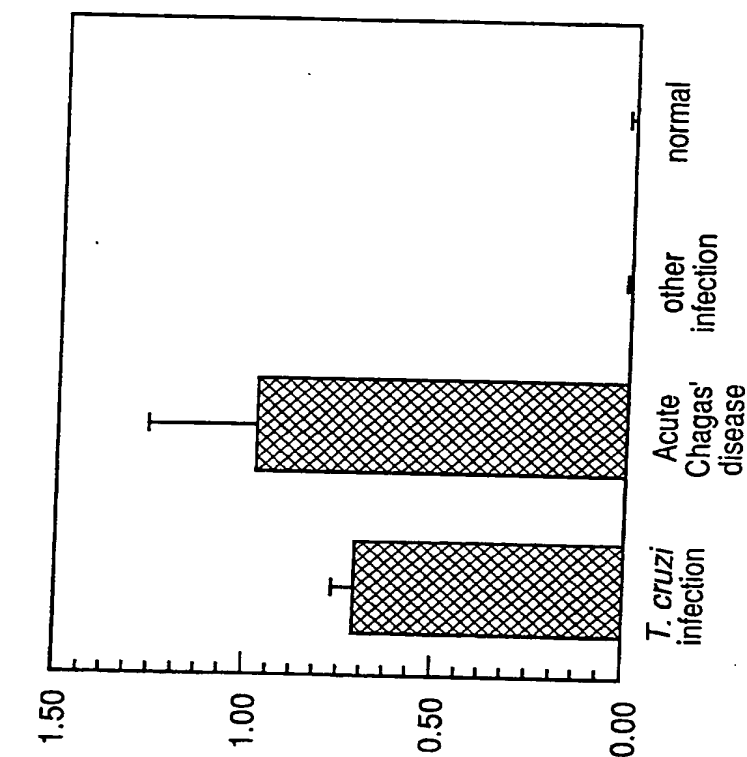


FIGURE 2B

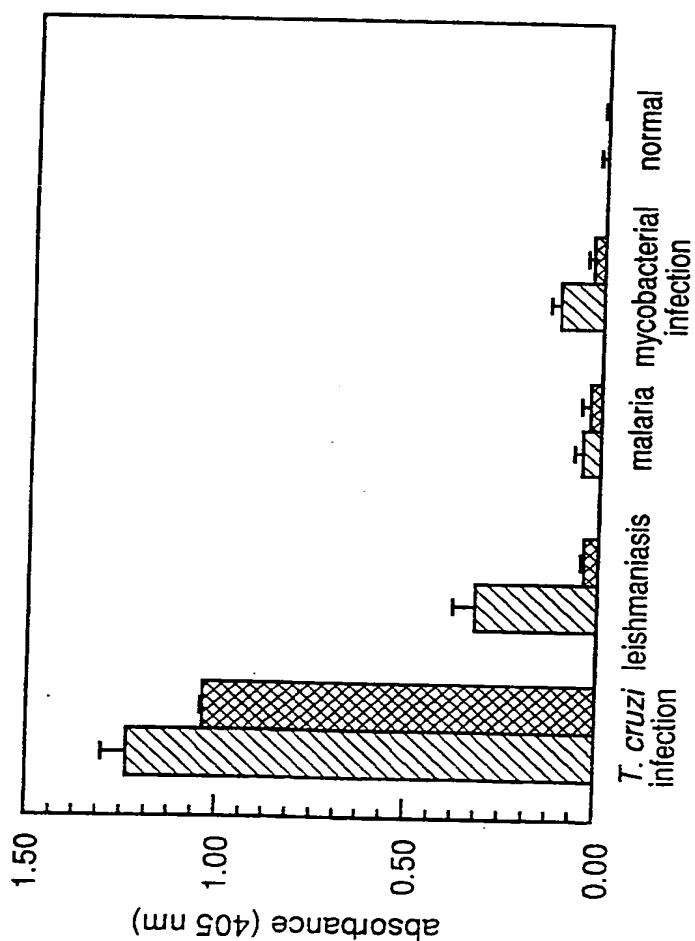


FIGURE 2A

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01231

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; A61K 39/00, 37/02

US CL :530/300; 424/88; 435/7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300; 424/88; 435/7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 234, issued 31 October 1986, Ouaisi et al, " <u>Trypanosoma cruzi</u> Infection Inhibited by Peptides Modeled from a Fibronectin Cell Attachment Domain", pages 603-607, see the Abstract.	1-3, 7,8
Y	Journal of Clinical Microbiology, Volume 29, No. 9, issued September 1991, Vergara et al, "Assay for Detection of <u>Trypanosomas cruzi</u> Antibodies in Human Sera Based on Reaction with Synthetic Peptides", pages 2034-2037, see entire document.	4-6

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art/
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

22 MARCH 1993

Date of mailing of the international search report

14 APR 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

H. SIDBERRY

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01231

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular and Biochemical Parasitology, Volume 48, issued 1991, Duncan et al, 'African Trypanosomes Express an Immunogenic Protein with a Repeating Epitope of 24 Amino Acids', pages 11-16, entire document.	1-3

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*